Biomass Production of Marine Microalgae as Feedstock for Bioenergy in Synthetic Urea Wastewater

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Abstract.. Growth rate of marine microalgae (*Nannochloropsis oculata, Tetraselmis chuii,* and *Porphyridium* sp.) in urea wastewater based medium is investigated. Microalgae were cultivated in sterile seawater under controlled conditions with varying amounts of urea: 0 mg/L (as a control variable), 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 150 mg/L. During the experiment, environmental parameters, including pH, temperature, dissolved oxygen (DO), and salinity, were monitored. The study showed that the microalgae cell growth rate of *Nannochloropsis oculata* was 0.44 x 10^4 cells/mL/day. While *Porphyridium sp.* and *Tetraselmis chuii* were found to be 0.61 x 10^4 cells/mL/day and 0.56 x 10^4 cells/mL/day. The optimum biomass productivity of microalgae *Nannochloropsis oculate*, *Porphyridium sp*. and *Tetraselmis chuii* was found to be day, respectively.

Keywords: Marine microalgae, Urea, Seawater, Biofuel.

1 Introduction

The global energy crisis has become a major focus of public discussions and government policies worldwide [1]–[4]. The need for more energy, combined with the high expense of fossil fuels and the adverse environmental impact of greenhouse gas emissions, has led to a search for creative and sustainable solutions [5][6]. Researchers have investigated alternative energy sources such as bioenergy, which produces biodiesel and is deemed more economically efficient, socially responsible, and environmentally friendly [7]. Studies on alternative solutions

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for bioenergy have included examining biodiesel products from fungi, castor plants, black soldier fly larvae, lignocellulose, and microalgae [7]–[11]. Given their high productivity levels, microalgae are viewed as a promising source of biodiesel [12].

Microalgae have potential as a source of energy for producing biofuels made from biomass [13]. As third-generation biofuels, microalgae offer advantages over other bioenergy products, including fast growth, resilience to diverse environments, and lipid content suitable for biodiesel [14]. Previous studies have investigated various types of microalgae, including *Micractinium sp.*, *Tetradesmus obliquus*, *Chlorella vulgaris*, *Dunaliella parva*, *Choricystis minor*, and *Monoraphidium contortum* [15]–[20]. However, many of these studies focused on freshwater microalgae, with limited research on marine microalgae. Notably, marine microalgae such as *Nannochloropsis oculata* have been found to have higher lipid content than freshwater microalgae like *Chlorella vulgaris* [21]. The study of marine microalgae's potential for biodiesel is crucial in reducing the pressure on diminishing freshwater resources.

The utilization of microalgae as biodiesel offers environmental benefits, including the reduction of nitrogen in wastewater [22]. Excessive use of urea, one of the most commonly used nitrogen fertilizers in agriculture, can pollute soil and water [23]. Microalgae can absorb nitrogen, including urea, so selecting the right strain is critical in wastewater treatment [24], [25]. Therefore, employing microalgae for biodiesel production can help absorb nitrogen in the environment, supplementing their use as a renewable energy source.

Marine microalgae, such as *Nannochloropsis oculata*, *Tetraselmis chuii*, and *Porphyridium* sp., have shown great promise for biodiesel production due to their ability to produce high yields of biomass [26], [27]. To maximize their biodiesel potential and minimize urea waste, it is crucial to test the cultivation of these microalgae under synthetic urea waste pressure. The objective of this study is to investigate the growth rate and biomass yield of three marine microalgae species, namely *Nannochloropsis oculata*, *Tetraselmis chuii*, and *Porphyridium* sp. using synthetic urea waste water media.

2 Method

2.1 Microalgae Cultivation

The study employed *Nannochloropsis oculata* (A), *Tetraselmis chuii* (B), and *Porphyridium sp*. (C) strains of microalgae, obtained from the microalgae cultivation collection located at the Center for Marine Aquaculture Fisheries in Lampung, Indonesia. Optical microscopy, combined with a hemocytometer and hand counter was used to count each strain of microalgae [28]. . The algae strains were diluted into sterile seawater to achieve a cell density of 30 x 10^4 cells/mL for *Nannochloropsis oculata*, 7 x 10^4 cells/mL for *Tetraselmis chuii*, and 3 x 10^4 cells/mL for *Porphyridium* sp.

2.2 Microalgae Cultivation

Algae strains were grown in 2L glass vials filled with 1 L sterile seawater medium. To aid in the adaptation of microalgae to the new environment, 10 mg/L of Triple Super Phosphate (TSP) fertilizer was added. All media and equipment were sterilized by autoclaving at 121℃ for 20 minutes prior to use to eliminate any external contaminants. During the experiment, we closely monitored environmental parameters including salinity (27.21±0.99 ppt), temperature (24.31±0.87 ℃), pH (6.26±0.43), and dissolved oxygen (7.76±0.41 mg/L).

2.3 Synthetic Urea addition

Synthetic urea (CH_4N_2O) Pro Analis was diluted in 1 L of distilled water using a multilevel dilution system. The urea concentration was divided into six concentrations, including 25 mg/L (T2), 50 mg/L (T3), 75 mg/L (T4), 100 mg/L (T5), 150 mg/L (T6) as the primary parameter and the control variable (0 mg/L, T1). Each treatment was repeated three times and averaged to obtain precise results. The difference in concentration was intended to observe the effect of urea wastewater media on the growth of microalgae in a laboratory setting.

2.4 Microalgae Kinetic Growth

Microalgal growth was assessed daily by counting cells in 1 mL of culture medium using a light microscope with hemocytometer and hand counter. This process was performed for each microalgae species observed, repeated three times, and then averaged. The equation used to calculate the specific growth rate (μ) of each species is as follows: [26]:

$$\mu = \frac{\ln(N) - \ln(No)}{t - to} \quad (1)$$

In this equation, μ (d-1) represents the specific growth rate during the exponential phase. N represents the cell density at time (t), and N0 represents the initial density during the exponential phase (t0). The doubling time (td) of cells was calculated to indicate the mean biomass generation time corresponding to the specific growth rate (μ) derived from the formula provided below [26]:

$$td = \frac{\ln 2}{\mu} \quad (2)$$

The dry weight of microalgae was measured at the start of cultivation and at the point of highest density during cultivation (H-exponential). To do this, 25 mL of media was filtered using GF/C filter paper (90 mm diameter) with the assistance of a 27 kPa vacuum pump. To avoid any moisture that could affect the mass of the filter paper, it was preheated in an oven at 105℃ for 1 hour before filtration. After filtration, the filter paper was reheated using the same method. Microalgae biomass productivity is determined by calculating the dry weight of microalgae using the following formula [17]:

$$\text{Biomass Yield} = \frac{(DLEP - DEEP)}{\Delta t}. \quad (3)$$

Where, DELP and DEEP represent the dry weight (g/L) at the end of the exponential period and the beginning of exponential growth.

2.5 Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) descriptive statistics. One-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$ was used to test daily cell density records for normality and heterogeneity. Tukey's posthoc test was used to compare cell density between different urea concentrations. Pearson's correlation test was used for examination of the relationship between variables.

3 Results and Discussion

3.1 Cell density

The study examined the effect of increasing urea concentrations (0 mg/L, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, and 150 mg/L) on the growth of the microalgae *Nannochloropsis oculata, Tetraselmis chuii,* and *Porphyridium* sp.. The batch cultivation had different observation times, with *Nannochloropsis oculata* taking 9 days to reach the desired growth and *Tetraselmis chuii* and *Porphyridium sp*. taking 7 days each Successful cultivation was indicated by the absence of significant growth inhibition during the exponential phase. This phase involves sequential cell division, resulting in an increase in the specific growth rate that continues until it reaches its peak value [29]. This confirms the adaptability of microalgae in the early phase to the urea-polluted environment.

The growth density of *Nannochloropsis oculata* cells during the exponential phase cultivated for 9 days was found to vary with each experiment **(Figure 1-A).** The experimental strains (A1, A2, A3, A4, A5, A6: initial density of 300 x 104 cells/mL) experienced the highest growth at different densities and times, respectively at 2990 x 10^4 cells/mL (Day 5), 4350 x 10^4 cells/mL (Day 6), 4475 x 10^4 cells/mL (Day 7), 4531 x 10^4 cells/mL (Day 7), 4480 x 10^4 cells/mL (Day 7), and 4805 x 10^4 cells/mL (Day 7). Treatment A6 (150 mg/L urea) showed the highest cell density growth of 4805 x 10^4 cells/mL on day 7. In contrast to the *Tetraselmis chuii* strain, which only requires a cultivation period of 7 days (**Figure 1-B),** each treatment (B1, B2, B3, B4, B5, B6: initial density of 30 x 10^4 cells/mL) reached its optimum density at different times: 282 x 10^4 cells/mL (Day 5), 383 x 10^4 cells/mL (day 5), 493 x 10^4 cells/mL (day 5), 571 x 10^4 cells/mL (day 6), 577 x 10^4 cells/mL (day 6), and 623 x 10^4 cells/mL (day 6), respectively. Treatment A6 (150 mg/L urea) also showed the highest density value of 623 x 10^4 cells/mL (day 6). In contrast, the *Porphyridium* sp strain, cultivated for 7 days at an initial cell density of 70 x 104 cells/mL, showed a different optimum cell density **(Figure 1-C).** The optimum cell density for each

treatment was 529 x 10^4 cells/mL (C1; day 5), 729 x 10^4 cells/mL (C2; day 5), 2335 x 10^4 cells/mL (C3; day 6), 2480 x 10^4 cells/mL (C4; day 6), 3037 x 10^4 cells/mL (C5; day 6), and 3787 x 10^4 cells/mL (C6; day 6). The treatment with the highest cell density was C6, which had 150 mg/L urea added on day 6, with a value of 3787 x 10^4 cells/mL.

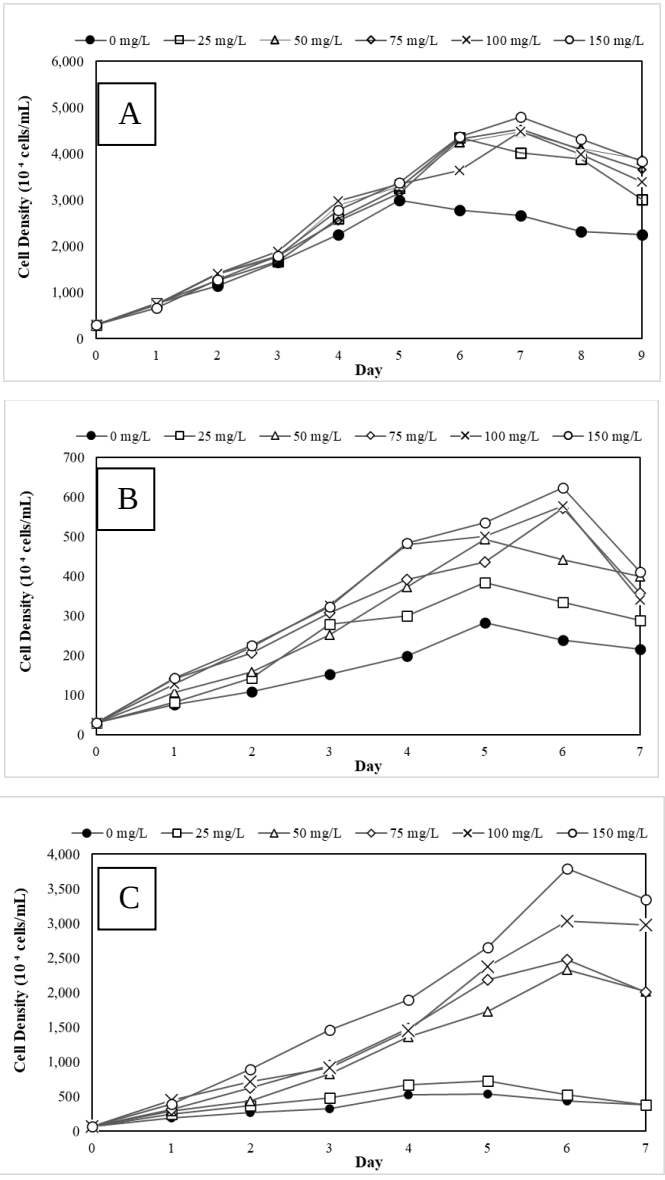

Fig. 1. Microalgae cell density (10^4 cells/mL). A) *Nannochloropsis oculata*; B) *Tetraselmis chuii*; C) *Porphyridium sp.*

The study results show that adding urea affects the growth of cell density in each microalgae strain. Nannochloropsys oculata strain (treatment A6) experienced 1.6 times more cell density growth than non-urea treated cells (A1). *Tetraselmis chuii* and *Porphyridium sp* strains both experienced significant increases in cell density growth compared to the control variable. Specifically, *Tetraselmis chuii* saw a growth of 2.21 times the cell density of the control, while *Porphyridium sp* saw a growth of 7.02 times. The statistical tests (one-way ANOVA) for each species revealed no significant difference in the provision of urea concentrations between *Nannochloropsis oculata* and *Tetraselmis chuii* species ($p=0.25>0.05$ and $p=0.796>0.05$). Therefore, it can be assumed that urea did not have a major influence on the growth of these two types of microalgae. In *Porphyridium sp*., the application of urea with varying concentrations significantly affects microalgae cell growth ($p=0.01<0.05$). This finding is consistent with previous research showing that an enhancement in the growth of microalgae species, such as Coccomyxa acidophila, can be significantly increased by the addition of urea [30].

The addition of urea at 150 mg/L dominated the significant growth of microalgae density. This is because microalgae can utilize urea as a nitrogen source for nutrients [31]. Urea seems to promote better microalgae growth compared to ammonia [32]. However, a lack of available nitrogen can lead to nutrient deficiencies, resulting in a decrease in the growth rate of microalgae [28]. In this study, each control variable (without treatment) has the lowest density in each species, which is the defining condition. Consequently, increased cell density growth can thrive at higher urea concentrations (150 mg/L).

3.2 Specific growth rate (μ) and doubling time (dt)

The specific growth rates (μ) of the three algal species examined varied considerably **(Figure 2).** At a concentration of 150 mg/L, the highest values were recorded for all three species: 0.44 day^{-1}, 0.25 day^{-1}, and 0.76 day^{-1}, respectively, with doubling times (dt) of 1.5 days, 2.72 days, and 0.96 days **(Figure 3)**. Compared to the control variable, the specific growth rate only reached 0.12 day-1, 0.06 day-1, and 0.14 day-1. Compared to A. *carterae* tested in urea wastewater, Tetraselmis chuii had a lower μmax value of 0.30 ± 0.08 day-1 [33]. The assay determined that *Tetraselmis chuii* had a maximum growth rate (μmax) of 0.686 μmax/day under varying salinity conditions [34], [35]. This difference is believed to be primarily due to variations in nutrient availability which affects the response of microalgae to nutrient absorption in their environment and subsequently impacts growth rates [36].

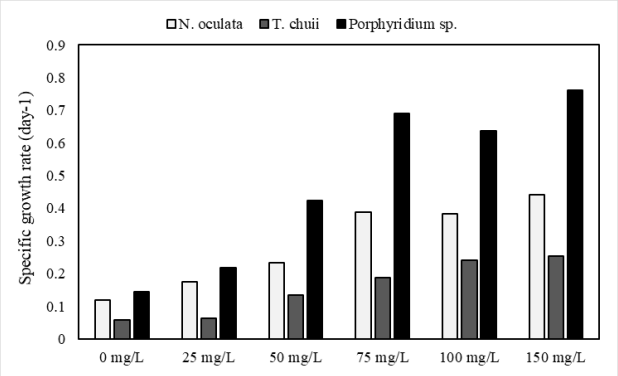

Fig. 2. Specific Growth Rate (day^{-1})

The doubling time analysis revealed that the *Tetraselmis chuii* species had the highest value compared to other species and concentrations. Specifically, the control variable and 25 mg/L urea concentration required 11.7 days and 10.9 days, respectively. The Pearson correlation showed a correlation (sig<0.05) of the specific growth rate with doubling time, resulting in a correlation coefficient of -0.745. Based on this, there is a strong inverted linear correlation between both variables.

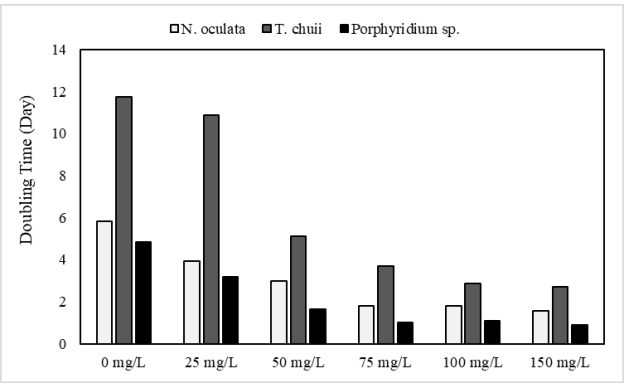

Fig. 3. Doubling Time (day)

3.3 Biomass Yield

The microalgae species evaluated (*Nannochloropsis oculata*, *Tetraselmis chuii*, and *Porphyridium* sp.). achieved their highest biomass yield rate at a urea concentration of 150 mg/L Specifically, the production rates were 821.33 g/m^3/day, 522.94 g/m^3/day, and 1244.4 g/m^3/day, respectively **(Figure 4).** A Pearson correlation analysis between the specific growth

rate and biomass production revealed a strong correlation (sig<0.05) with a correlation coefficient of 0.918. Environmental conditions such as light exposure, temperature variations, necessary nutrients, carbon dioxide availability, system pH, and salinity can influence the growth of microalgae, which is crucial for biodiesel fuel production [37]. The addition of urea at all replacement levels can increase protein, while the carbohydrate content in N. oc-ulata decreases. Microalgal species tend to have a decrease in carbohydrate amount as protein quantity increases [38]. Triglyceride accumulation in cells is higher with an increase in alkaline pH. Conversely, acidic pH can facilitate changes in nutrient uptake or create pollutants that are harmful to algal growth [39]. Environmental conditions affect nutrient absorption, including the addition of urea as a nutrient in the microalgae medium.

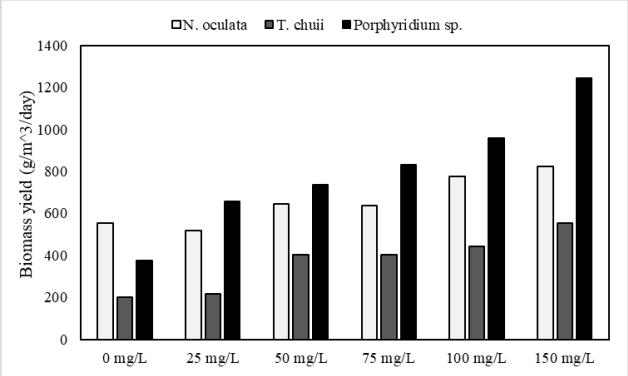

Fig. 4. Biomass yield (g/m^3/day)

The study was limited to biomass, and although lipids were not studied, the state of biomass can be used as a reference in biodiesel production. Previous research has shown that *Nannochloropsis oculata* has a biodiesel conversion percentage of 85.12% [40]. Our findings indicate that *Porphyridium sp* microalgae has higher biomass production than *Nannochloropsis oculata* and *Tetraselmis chuii*, making it a potential biodiesel feedstock. Additionally, due to its high biomass production, *Porphyridium sp* microalgae can also be used as a cosmetic raw material [41]. This makes this species potential as a biodiesel feedstock because it has high biomass production.

4 Conclusion

The research findings show that Nannochloropsis oculata microalgae had a growth rate of 0.44 x 10^4 cells/mL/day, while Porphyridium sp. and Tetraselmis chuii had growth rates of 0.61 x 10^4 cells/mL/day and 0.56 x 10^4 cells/mL/day, respectively. Additionally, Nannochloropsis oculata, Porphyridium sp., and Tetraselmis chuii had optimal biomass productivity rates of 82.3 g/m^3/day, 552.94 g/m^3/day, and 1244.4 g/m^3/day, respectively. The utilization of microalgae shows promise in mitigating the negative effects of urea on marine water pollution.

References

[1] Somoye O.A.: Energy crisis and renewable energy potentials in Nigeria: A review., Vol. 188, No. October. pp: 113794. Energy Rev.Renew. Sustain (2023)

[2] Corbos R.A., Bunea O.I., and Jiroveanu D.C.: The effects of the energy crisis on the energy-saving behavior of young people. Vol. 49, No. 2. pp: 101184. Energy Strateg. Rev. (2023)

[3] Caldera U., Gulagi A., Jayasinghe N., and Breyer C.: Looking island wide to overcome Sri Lanka's energy crisis while gaining independence from fossil fuel imports. Vol. 218, No. December 2022. pp: 119261. Renew. Energy (2023)

[4] Hille E.: Europe's energy crisis: Are geopolitical risks in source countries of fossil fuels accelerating the transition to renewable energy?.Vol. 127, pp: 107061. Energy Econ. (2023)

[5] Doğan B., Ghosh S., Shahzadi I., Balsalobre-Lorente D., and Nguyen C.P.: The relevance of economic complexity and economic globalization as determinants of energy demand for different stages of development. Vol. 190, pp: 371–384. Renew. Energy (2022)

[6] Shang Y., Han D., Gozgor G., Mahalik M.K., and Sahoo B.K.: The impact of climate policy uncertainty on renewable and non-renewable energy demand in the United States. Vol. 197, No. June. pp: 654–667. Renew. Energy (2022)

[7] Sundaramahalingam M.A. and Sivashanmugam P.: Biodiesel production from a novel renewable source through the ultrasound-assisted transesterification process, using energy efficient nanocatalyst developed from waste material. Vol. 346, No. January. pp: 128397. (2023)

[8] Qenawy M. et al.: Performance and emission of extracted biodiesel from mixed Jatropha-Castor seeds. Vol. 357, pp: 130060. Fuel (2024)

[9] Kathumbi L.K., Home, P.G. Raude J.M., and Gathitu B.B.: Performance and emission characteristics of a diesel engine fuelled by biodiesel from black soldier fly larvae: Effects of synthesizing catalysts with citric acid. Vol. 9, No. 11. pp: e21354. Heliyon (2023)

[10] Garg R., Sabouni R., and Ahmadipour M.: From waste to fuel: Challenging aspects in sustainable biodiesel production from lignocellulosic biomass feedstocks and role of metal organic framework as innovative heterogeneous catalysts. Vol. 206, No. August. pp: 117554. Ind. Crops Prod. (2023)

[11] Ahn Y. and Kim J.: Economic design framework of microalga-based biodiesel supply chains under uncertainties in CO2 emission and diesel demand. Vol. 155, pp: 107538. Comput. Chem. Eng. (2021)

[12] Casanova L.M. et al.: Development of Microalgae Biodiesel: Current Status and Perspectives. Vol. 11, No. 1. pp. 1–20. Microorganisms (2023)

[13] Tien Thanh N. et al.: Fundamental understanding of in-situ transesterification of microalgae biomass to biodiesel: A critical review. Vol. 270, No. September. pp: 116212. Bioresour. Technol. (2022)

[14] Díaz V., Leyva-Díaz J.C., Almécija M.C., Poyatos J.M., del Mar Muñío M., and Martín-Pascual J.: Microalgae bioreactor for nutrient removal and resource recovery from wastewater in the paradigm of circular economy. Vol. 363, No. September. Bioresour. Technol. (2022)

[15] Sorokina K.N., Samoylova Y.V., Gromov N.V., Ogorodnikova O.L., and Parmon V.N.: Production of biodiesel and succinic acid from the biomass of the microalga Micractinium sp. IC-44. Vol. 317, No. July. pp: 124026. Bioresour. Technol. (2020)

[16] Koley S., Sonkar S., Bagchi S.K., Patnaik R., and Mallick N.: Development of a low-cost cultivation medium for simultaneous production of biodiesel and bio-crude from the chlorophycean microalga Tetradesmus obliquus: A renewable energy prospective. Vol. 364, No. October 2019. pp: 132658. J. Clean. Prod. (2022)
[17] Almarashi J.Q.M., El-Zohary S.E., Ellabban M.A., and Abomohra A.E.F.: Enhancement of lipid production and energy recovery from the green microalga Chlorella vulgaris by inoculum pretreatment with low-dose cold atmospheric pressure plasma (CAPP). Vol. 204, No. November 2019. pp: 112314. Energy Convers. Manag. (2020)
[18] Fawzy M.A. and Alharthi S.: Use of Response Surface Methodology in optimization of biomass, lipid productivity and fatty acid profiles of marine microalga Dunaliella parva for biodiesel production. Vol. 22, pp: 101485. Environ. Technol. Innov. (2021)
[19] Oliveira C.Y.B., D'Alessandro E.B., Antoniosi Filho N.R., Lopes R.G., and Derner R.B.: Synergistic effect of growth conditions and organic carbon sources for improving biomass production and biodiesel quality by the microalga Choricystis minor var. minor. Vol. 759, pp: 143476. Sci. Total Environ. (2021)
[20] Pineda-Camacho G., de M. Guillén-Jiménez F., Pérez-Sánchez A., Raymundo-Núñez L.M., and Mendoza-Trinidad G.: Effect of CO2 on the generation of biomass and lipids by Monoraphidium contortum: A promising microalga for the production of biodiesel. Vol. 8, No. August. Bioresour. Technol. Reports (2019)
[21] Aliyu A., Lee J.G.M., and Harvey A.P.: Microalgae for biofuel: Isothermal pyrolysis of a fresh and a marine microalga with mass and energy assessment. Vol. 14, No. March pp: 100474. Chem. Eng. J. Adv. (2023)
[22] Nakarmi K.J., Daneshvar E., Mänttäri M., and Bhatnagar A.: Removal and recovery of nutrients from septic tank wastewater using microalgae: Key factors and practical implications. Vol. 345, No. August. J. Environ. Manage. (2023)
[23] Xu X., Ma F., Zhou J., and Du C.: Control-released urea improved agricultural production efficiency and reduced the ecological and environmental impact in rice-wheat rotation system: A life-cycle perspective. Vol. 278, No. June 2021. pp: 108445. F. Crop. Res. (2022)
[24] Kumar A. and Bera S.: Revisiting nitrogen utilization in algae: A review on the process of regulation and assimilation. Vol. 12, No. October. pp: 100584. Bioresour. Technol. Reports (2020)
[25] Ross M.E., Davis K., McColl R., Stanley M.S., Day J.G., and Semião A.J.C.: Nitrogen uptake by the macro-algae Cladophora coelothrix and Cladophora parriaudii: Influence on growth, nitrogen preference and biochemical composition. Vol. 30, No. December 2017. pp: 1–10. Algal Res. (2018)
[26] Kholssi R., Riouchi O., Douhri H., and Debdoubi A.: Microalgae as a sustainable energy source: Growth and lipids production of Nitzschia sp., Nannochloropsis sp., and Tetraselmis sp. from Mediterranean seawater. Vol. 50, No. May. pp: 1–8. Biocatal. Agric. Biotechnol. (2023)
[27] Chhandama M.V.L., Ruatpuia J.V.L., Ao S., Chetia A.C., Satyan K.B., and Rokhum S.L.: Microalgae as a sustainable feedstock for biodiesel and other production industries: Prospects and challenges. Vol. 12, No. October. pp: 100255. Energy Nexus (2023)
[28] Morales-Plasencia, M.E., Ibarra-Castro L., Martínez-Brown J.M., Nieves-Soto M., Bermúdez-Lizárraga J.F., and Rojo-Cebreros A.H.: The effect of nitrogen limitation on carbohydrates and β-glucan accumulation in Nannochloropsis oculata. Vol. 72, No. April. Algal Res. (2023)
[29] Abdullahi Z.H., Marselin F.N., Khaironizam N.I.A., Fauzi N.F.A., and Wan Maznah W.O.: Growth stage-related biomass, pigments, and biochemical composition of Stichococcus bacillaris, Synechococcus sp., and Trentepohlia aurea isolated from Gua Tempurung, a cave in Malaysia. Vol. 197, No. June 2022. pp: 107633. Plant Physiol. Biochem. (2023)
[30] Casal C., Cuaresma M., Vega J.M., and Vilchez C.: Enhanced productivity of a lutein-enriched novel acidophile microalga grown on urea. Vol. 9, No. 1. pp: 29–42. Mar. Drugs (2010)
[31] Sun C., Xia A., Liao Q., Fu Q., Huang Y., and Zhu X.: Life-cycle assessment of biohythane production via two-stage anaerobic fermentation from microalgae and food waste. Vol. 112, No. May. pp: 395–410. Renew. Sustain. Energy Rev. (2019)
[32] Song X., Liu B.F., Kong F., Song Q., Ren N.Q., and Ren H.Y.: Lipid accumulation by a novel microalga Parachlorella kessleri R-3 with wide pH tolerance for promising biodiesel production.

Vol. 69, No. July 2022. pp: 102925. Algal Res. (2023)
[33] Molina-Miras A.: A step forward in sustainable pesticide production from Amphidinium carterae biomass via photobioreactor cultivation with urea as a nitrogen source. Vol. 387, No. June. Bioresour. Technol. (2023)
[34] Haris N., Manan H., Jusoh M., Khatoon H., Katayama T., and Kasan N.A.: Effect of different salinity on the growth performance and proximate composition of isolated indigenous microalgae species. Vol. 22, pp: 100925. Aquac. Reports (2022)
[35] Yaashikaa P.R., Kumar P.S., and Karishma S.: Bio-derived catalysts for production of biodiesel : A review on feedstock, oil extraction methodologies, reactors and lifecycle assessment of biodiesel. Vol. 316, No. December 2021. pp: 123379. Fuel (2022)
[36] Tang C et al.: Bioresource Technology Responses of microalgae under different physiological phases to struvite as a buffering nutrient source for biomass and lipid production. Vol. 384, No. June. pp: 129352. Bioresour. Technol. (2023)
[37] Shiong K., Ahmad I., Wayne K., Iwamoto K., and Bhatnagar A.: Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae : A review. Vol. 96, No. February. pp: 101071. Prog. Energy Combust. Sci. (2023)
[38] Otero A. and Fábregas J.: Changes in the nutrient composition of Tetraselmis suecica cultured semicontinuously with different nutrient concentrations and renewal rates. Vol. 159, No. 1–2, pp: 111–123. Aquaculture (1997).
[39] Foteinis S., Chatzisymeon E., Litinas A., and Tsoutsos T.: Used-cooking-oil biodiesel : Life cycle assessment and comparison with fi rst- and third-generation biofuel. Vol. 153, pp: 588–600. Renew. Energy (2020)
[40] He Y., Zhang B., Guo S., Guo Z., Chen B., and Wang M.: Sustainable biodiesel production from the green microalgae Nannochloropsis: Novel integrated processes from cultivation to enzyme-assisted extraction and ethanolysis of lipids. Vol. 209, No. December 2019. pp: 112618. Energy Convers. Manag. (2020)
[41] Mourelle M.L.: The Potential Use of Marine Microalgae and Cyanobacteria in Cosmetics and Thalassotherapy. Cosmetics, (2017)